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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/823,784
Filing Date: April 14, 2004
Appellant(s): UHLMANN ET AL.

Joyce von Natzmer
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed January 25, 2010 appealing from the Office action mailed January 23, 2009.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

No amendment after final has been filed.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

6258568	Nyren	7-2001
5786146	Herman	7-1998
7078168	Sylvan	7-2006
5602000	Hyman	2-1997
US 2002/0086324 A1	Laird	7-2002
US 2003/0232351 A1	Feinberg	12-2003

Uhlmann, Karen et al. "Changes in methylation patterns identified by two-dimensional DNA fingerprinting" Electrophoresis Vol 20 (1999) pages 1748-1755

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

A. Claims 1-5, 7-9, 11-12, 19-20, 22-24, 26-33, and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001).

Regarding Claims 1, 7, 9, 12, 22, 24, 27, 31-33, and 36 Uhlmann teaches a method for identifying methylated cytosines comprising treating a sample containing genomic DNA derived from blood and tumor tissue with sodium bisulfite and amplifying the sample by PCR. Uhlmann et al further teach that the amplified nucleic acids were then cloned and plasmid DNA of the clones was prepared and sequenced using the

dideoxynucleotide chain termination method determine the methylation state of the amplified product (Page 1750-1751). Thus Uhlmann teaches treating a nucleic acid sample with an agent suitable for the conversion of a nucleotide if present in methylated or non methylated form to pair with a nucleotide normally not pairing with the nucleotide prior to conversion, and amplifying the nucleic acid, sequencing the nucleic acid, and detecting whether said nucleotide is methylated. It is noted that the phrase in claim 12 "to diagnose said pathological condition or the predisposition for said pathological condition" is an intend use and does not limit the method steps. Even if the claims were amended to actually require a "diagnosing" step they would still be rejected because Uhlmann teaches an association between hypomethylation and pilocytic astrocytomas.

Uhlmann does not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules. Uhlmann does not teach a method wherein said amplification primer is labeled with a biotin. Further Uhlmann does not teach that the amplified nucleic acids were sequenced using a real-time sequencing method that comprises hybridizing a sequencing primer to a single stranded nucleic acid, adding a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine phosphosulfate (APS), and luciferin, sequentially adding each dNTP, and detecting a luminescent signal. Further Uhlmann does not teach a sequencing method that is a high throughput method.

However Nyren teaches an alternative method for sequencing. The method of Nyren PCR is performed using one or more primers that carry a functional group such as a biotin which permits subsequent immobilization and aids in the separation of a

single stranded DNA (Col 8, lines 1-5). Thus Nyren teaches a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules. Nyren further teaches a real time sequencing method called pyrosequencing that can be used to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are mixed with a DNA polymerase, a ATP sulfurylase, a luciferase, a apyrase, a adenosine phosphosulfate and luciferin. Then dNTPs are successively added to the same sample primer mixture and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (Column 2, lines 25-42). Thus Nyren teaches a method wherein the amplified nucleic acids are sequenced using a high throughput real-time sequencing method. It is a property of the method of Nyren that the identity of more than one nucleotide is determined. Nyren also teaches that pyrosequencing can be used to detect disease.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann by using the sequencing method of Nyren which includes performing PCR with at least one amplification primer labeled with a biotin and then sequencing the single stranded nucleic acid via pyrosequencing. Specifically Nyren teaches that amplification primer with labels that form anchors are useful for the removal of single stranded amplified

nucleic acid molecules. Further Nyren et al teaches that the benefit of performing pyrosequencing over other sequencing methods such as the enzymatic chain termination method of Sanger is that pyrosequencing enables a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels (Column 1, lines 60-64). Nyren further teach that other sequencing methods which rely on electrophoresis are not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1, lines 15-30). However the pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous measurement of the progress of the polymerization reaction with time. The method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6). Further the claimed method is obvious because the substitution of the PCR, cloning, and sequencing steps performed by Uhlmann for the PCR and sequencing steps performed by Nyren would have yielded predictable results to one or ordinary skill in the art at the time of the invention.

Regarding Claims 2-4, 19, and 28-29 Uhlmann teaches a method wherein the nucleic acid sample is genomic DNA derived from peripheral blood lymphocytes and from tumor tissue (page 1749, col 1). Thus Uhlmann teaches a method wherein the sample DNA is derived from a tumor tissue and a body fluid.

Regarding Claims 5 and 20 Uhlmann teaches a method wherein the nucleic acid is amplified via PCR (page 1749, col 1).

Regarding Claims 8, 11, 23, 26, and 30 Uhlmann teaches a method wherein non methylated cytosines are converted to uracil via sodium bisulfite treatment (page 1749, col 1). Thus Uhlmann teaches a method wherein the nucleotide of (a) is a cytosine and is part of a CpG. Upon bisulfite treatment the methylated cytosines are converted to uracils so that they pair with an adenosine instead of a guanine.

B. Claims 12-16, 18 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001) and in further view of Herman (U.S. Patent 5786146 Issued 1998).

The teachings of Uhlmann et al and Nyren et al are presented above.

The combined references do not teach that the methylation status is used to diagnose a pathological condition such as cancer, a neurodegenerative disease or another neurological disorder. The combined references also do not teach that the methylation status is used diagnose cancer that is a primary tumor, a metastasis or a residual tumor. The combined references do not teach that the primary tumor is a glioma selected from the group comprising: astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, and a pilocytic astrocytoma. The combined references also do not teach that the neurological disorder is selected from the group comprising: Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

However, Herman et al teaches that the detection of methylated CpG containing nucleic acid is indicative of several disorders. Such disorders include but are not limited to low grade astrocytoma, anaplastic astrocytoma, glioblastoma, medulloblastoma, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, endometrial cancer and neuroblastoma. Identification of methylated CpG status is also useful for detection and diagnosis of genomic imprinting, fragile X syndrome and X-chromosome inactivation (Column 10, lines 49-58).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Uhlmann and Nyren to diagnose the pathological disorders that Herman teaches that are associated with methylation. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of several pathological disorders. Accordingly, one of ordinary skill in the art would have been motivated to use the method of Uhlmann and Nyren in order to have achieved the advantage of being able to diagnose these diseases.

C. Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) and Herman (U.S. Patent 5786146 Issued 1998) as applied to claims 12 and 38 above, and in further view of Feinberg (Pub No. US 2003/0232351).

The teachings of Uhlmann, Nyren, and Herman are presented above.

The combined references do not teach a method used to diagnose neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

However, Feinberg teaches a method of determining a disease state in a subject by determining DNA methylation status. Although the disease state is often cancer, the methods taught by Feinberg also include Alzheimer's disease and Parkinson's disease (Paragraph 0029).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Uhlmann, Nyren and Herman used to diagnose primary tumors, to also diagnose neurodegenerative diseases. It was well known in the art at the time the invention was made that the detection of methylated sequences is indicative of certain neurodegenerative diseases. Accordingly, one of ordinary skill in the art would have been motivated to use the method of Uhlmann, Nyren and Herman in order to have achieved the advantage of being able to diagnose these diseases.

D. Claims 10, 25, 34, and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claims 1 and 12 above, and in further view of Sylvan (US Patent 7078168 Filed 2/2002).

The teachings of Uhlmann and Nyren are presented above.

The combined references do not teach a method further comprising calculating a frequency of methylated nucleotides from the results of said real time sequencing. Further the combined references do not teach a method wherein an allele frequency of 5% can be detected or a method wherein an allele frequency of 5% with a standard deviation of not more than 1% is detected. In the instant case the allele frequency is being interpreted as a range of 4%-6%.

However, Sylvan teaches a method of determining the frequency of an allele in a population of nucleic acid molecules. The method comprises performing primer extension reactions using a primer which binds at a predetermined site located in nucleic acid molecules and obtaining a pattern of nucleotide incorporation (Abstract). Specifically Figs 4a-c and Fig 6-7 depicts graphically relative peak heights from a pyrosequencing reaction plotted against allele frequency. As you can see an allele frequency was detected. The wherein clause in claim 34 is conditional in view of the "can be" language. Therefore the claims actually only require detecting an allele frequency. Regarding claim 39, Sylvan does not exemplify a method wherein an allele frequency of 5% was detected with a standard deviation of not more than 1%, however Sylvan teaches that for SNPs SNPE1.5, SNPE7.5 and SNPE4.5 (See Figs 4a-c) it is expected that allele frequencies of 5% with a standard deviation of not more than 1% can be detected. Therefore even if the expected results do not end up being equivalent to the obtained results, it would be obvious to modify the method in order to determine an allele frequency of 5% with a standard deviation of not more than 1%.

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann and Nyren by further calculating the frequency of methylated nucleotides from the results of the pyrosequencing as suggested by Sylvan. The method of Sylvan is advantageous in that it determines the exact sequence of a nucleic acid fragment while directly measuring the amount of nucleotide incorporated. Using this method is it possible to obtain accurate, cost effective, and rapid information on allele frequencies (Column 22, lines 39-67 and Column 23, lines 1-4).

E. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claim 1 above, and in further view of Laird (US 2002/0086324 Filed 10/2001).

The teachings of Uhlmann and Nyren are presented above.

The combined references do not teach a method wherein the amplification primer does not comprise CpG.

However, Laird teaches a method wherein a genomic DNA is provided that has mixed methylation status. The sample is converted in a standard sodium bisulfite reaction and the mixed products are amplified by a PCR reaction using primers that do not overlap any CpG dinucleotides. This produces an unbiased (with respect to methylation status) heterogeneous pool of PCR products. The mixed or heterogeneous

pool can then be analyzed by a technique capable of detecting sequence differences (Para 0037).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann and Nyren by using an amplification primer that does not contain CpGs as suggested by Laird. The method of Laird is advantageous because primers that lack CpG dinucleotides can be used to amplify the sequence between the two primers, regardless of the DNA methylation status of that sequence in the original genomic DNA (Para 0016).

F. Claim 37 is rejected under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claims 1 and 8 above, and in further view of Hyman (US 5602000 1997).

The teachings of Uhlmann and Nyren are presented above.

The combined references do not teach a method wherein said nucleotide of claim 1 step (a)(i) is an adenine.

However Hyman teaches a method wherein adenine is converted to hypoxanthine to give rise to a nucleotide pairing with cytosine (Example 19).

Accordingly it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann and Nyren by converting adenine to hypoxanthine to give rise to a nucleotide pairing with cytosine as

suggested by Hyman. Methods of converting adenine to hypoxanthine were well known in the art at the time of the invention as demonstrated by Hyman and thus one could have combined the methods of Uhlmann, Nyren, and Hyman and the results would have been predictable to one of skill at the time of the invention.

(10) Response to Argument

A. Rejection of claims 1-5, 7-9, 11-12, 19-20, 22-24, 26-33, and 36 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001).

In the brief the Appellants summarize the teachings of Uhlmann which is the primary reference (pages 10-12 of the brief). Additionally the Appellants summarize the teachings of Nyren which is the secondary reference (page 13 of the brief).

The first argument presented in the brief is that the prior art does not teach each element claimed.

Regarding claim 32 the Appellants state that claim 32 requires "determining the amount of said nucleotide pairing with said new nucleotide pairing partners". The Appellants argue that the Examiner has not provided any showing for this element (see page 14 of the brief).

This argument has been full considered but is not persuasive. In the instant case Nyren teaches a real time sequencing method called pyrosequencing that can be used

to identify a base at a predetermined position in a DNA sample using an extension primer, which hybridizes immediately adjacent to the target position. The DNA sample and extension primer are mixed with a DNA polymerase, a ATP sulfurylase, a luciferase, a apyrase, a adenosine phosphosulfate and luciferin. Then dNTPs are successively added to the same sample primer mixture and the dNTPs will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position. When the PPi is released a certain amount of light gets released that is equivalent to the amount of incorporated nucleotides. The unincorporated dNTPs get degraded (column 2, lines 25-42, Col 5, lines 19-23). Since Nyren teaches determining the amount of incorporated nucleotides which are base pairing with the DNA strand that is being sequence, Nyren teaches a step of determining the amount of nucleotide pairing with the nucleotide pairing partners. The nucleotide pairing partners are considered new because the DNA strand was treated with bisulfite which converted the non methylated cytosines to uracil.

Regarding claims 1 and 12 the Appellants state that the claims require that treatment of the sample, e.g., with bisulfite takes place in “an aqueous solution”. They argue that the specification (page 6 lines 6 to 9 and lines 20 to 23) clarifies that an “aqueous solution” may be water such as distilled water, a buffered solution such as a phosphate buffered solution or buffered solution other than a phosphate buffered solution. The Appellants state that the bisulfite treatment in Uhlmann takes place in agarose beads and that the DNA remains in the beads until after the PCR reaction takes place (see page 14 of the brief).

These arguments have been fully considered but are not persuasive. Step (a) of claims 1 and 12 recites “treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide”. Uhlmann teaches that “About 100 ng denatured DNA (5 min, 95ºC) in 1.7% low melting agarose (Sigma) were dropped into chilled mineral oil to form agarose beads [19]. The fixed single stranded DNA was subjected to bisulfite treatment (2.5 M sodium metabisulfite, 125 mM hydroquinone, pH 5.0) for 4 h at 50ºC.” Because the nucleic acid molecule is embedded in an agarose bead and the agarose is in a solution of mineral oil, the nucleic acid molecule is technically in an aqueous solution. Additionally the bisulfite treatment itself is an aqueous solution so once its added, the nucleic acid molecule is again technically in an aqueous solution. Further Figure 1 of Uhlmann depicts the beads in an aqueous solution. Thus Uhlmann teaches treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide.

The examiner acknowledges that the specification (page 6 lines 6 to 9 and lines 20 to 23) provides examples of an “aqueous solution”, however a complete and fixed definition for this phrase has not been provided. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Since the claims are not limited to any specific type of "aqueous solution" the phrase has been given its broadest reasonable interpretation.

The second argument in the brief is that the substitution of PCR, cloning, and sequencing steps performed by Uhlmann for the PCR and sequencing steps performed by Nyren would not have yielded predictable results.

The Appellants argue that substituting the steps of Uhlmann for the steps of Nyren would leave the DNA of Uhlmann in the agarose beads and that this raises the question as to whether the relative complex sequencing reaction of Nyren that follows his PCR could be performed in such an environment. The Appellants state that the Examiner did not make clear why predictable results should be expected by employing a PCR using detectably labeled amplification primers for subsequent real time sequencing of a DNA sample that is contained in agarose beads. From the teachings of Uhlmann the Appellants state that one of skill in the art would be under the impression that the PCR amplification product would need to be gel extracted for further processing, in particular sequencing. The Appellants note that an additional step of a gel extraction would be at odds with the identified advantages (speed etc), that according to the Office would cause the person skilled in the art to combine the references (see page 15 of the brief).

These arguments have been fully considered but are not persuasive. First of all it is noted for the record that the claims do not require that the DNA is in an agarose bead, that the DNA is amplified while in an agarose bead, or that the DNA is extracted from an agarose bead. However one of skill in the art following the method of Uhlmann up to but not including the PCR step would end up with DNA in an agarose bead. Nyren teaches that the sample DNA (the DNA that is sequenced via pyrosequencing)

may be provided by any desired source of DNA, including for example PCR or other amplified fragments (Col 7, lines 65-67). Thus based on the this teaching the sample DNA may be provided from ANY source including an agarose bead that contains DNA amplified with a primer labeled with biotin. The Appellants have not provided any evidence that the amplified DNA in the agarose bead could not be used for pyrosequencing. However even if they did provide such evidence one of skill in the art at the time of the invention would have known how to extract the amplified DNA from the embedded bead prior, particularly since Uhlmann teaches that this can be done using the Qiaquick kit made by Qiagen. Thus the predictable result of determining the nucleic acid sequence of a particular DNA strand could be expected by performing a PCR reaction using detectably labeled amplification primers, extracting the amplified DNA from the embedded bead, and then subjecting the amplified DNA to real time sequencing. The argument that an additional step of removing the DNA from the bead would be at odds with the identified advantages (speed etc) is misleading because even if this step was performed the method of Nyren still has the advantages of enabling a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels (Column 1, lines 60-64). Further Nyren teaches that other sequencing methods which rely on electrophoresis are not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1, lines 15-30). The pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous

measurement of the progress of the polymerization reaction with time. Further the method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6).

Next the Appellants argue that Uhlmann's amplification primers are not detectably labeled and that Uhlmanns amplification product is, after gel extraction, cloned to produce single stranded DNA. The Appellants note that a person skilled in the art would be reluctant to make the modification to Uhlmann's amplification primers because it would interfere with the subsequent cloning step. The Appellants refer to US Patent 6,589,736 which states that "PCR products that are biotinylated are not suitable material for cloning" (col 7, line 23). The same patent further states that "the presence of biotin on the nascent DNA can interfere with its subsequent utilization in cloning or hybridization analysis" (col 34, line 40). Thus the Appellants submit that the modification of using biotinylated primers would render Uhlmann unsatisfactory for its intended purpose. The Appellants refer to MPEP 2143.01 and In re Gordon 733 F. 2d 900, 221 USPQ 1125 (Fed. Cir. 1984) for support. Additionally they refer to In re Ratti, 270 F.2d 810, 813 (CCPA 1959) and argue that the Examiners analysis changes the basic principles under which Uhlmann was designed to operate (see pages 16 and 17 of the brief).

These arguments have been fully considered but are not persuasive. Uhlmann is directed to analyzing changes in methylation patterns between tumor DNA and non tumor DNA which is accomplished by treating genomic DNA with sodium bisulfite and amplifying the sample by PCR. Then the amplified nucleic acids are cloned and

plasmid DNA from the clones is sequenced using the dideoxynucleotide chain termination method to determine the methylation state of the amplified product (Page 1750-1751). Modifying Uhlmann by performing PCR using at least one amplification primer that is detectably labeled with biotin and then sequencing the PCR products via pyrosequencing as suggested by Nyren would not require a step of cloning to detect methylation. Therefore the issue of Uhlmann being inoperable is not relevant. The modification of Uhlmann is not just the use of the biotinylated PCR primer. In the instant case the modification of Uhlmann is the use of the biotinylated PCR primer and pyrosequencing which replaces the PCR, cloning, and dideoxy sequencing steps of Uhlmann. The Appellants should recognize that it is not that one step of Uhlmann is being modified (i.e. the PCR step with the biotinylated primer) and then the rest of Uhlmann is being carried out. In actuality the PCR, cloning, and dideoxy sequencing steps of Uhlmann are being replaced by the PCR and pyrosequencing steps of Nyren. The modified method of Uhlmann in view of Nyren will comprise treating a sample with bisulfite (Uhlmann), performing PCR using a biotinylated primer (Nyren), and pyrosequencing (Nyren).

The third argument in the brief is that the advantages described by Nyren and considered relevant in the obviousness analysis would not motivate the person skilled in the art to combine the teachings of Uhlmann and Nyren.

The Appellants state that Uhlmann tried to obtain verification that the differences in 2-D-fingerprinting spots of tumor and non-tumor DNA are in fact a result of changed methylation, a task that requires primarily precision. The prospect of an automatic

approach for large scale, non-electrophoretic sequencing procedures which allow for continuous measurements, handling of multiple sample at the same time as described by Nyren, would be, if at all, at best be of secondary importance. The Appellants state that Nyren himself notes some issues with his method that could affect precision and thus discourage usage in methods that involves a high degree of precision (see col 7, lines 15). Furthermore, Nyren also makes clear that accumulation of reaction by-products may take place. While the problem can be avoided by periodic washing, it also adds reluctance if precision is the primary goal as in Uhlmann.

These arguments have been fully considered but are not persuasive. The argument that the primary goal of Uhlmann is precision is not persuasive because the Uhlmann paper does not even mention precision. Contrary to the assertion that there is no motivation to combine Uhlmann and Nyren, it is noted that Nyren teaches several benefits of pyrosequencing. For example Nyren specifically teaches that the benefit of performing pyrosequencing over other sequencing methods such as the enzymatic chain termination method of Sanger is that pyrosequencing enables a base to be identified in a target position and DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and use of harmful radiolabels (Column 1, lines 60-64). Nyren further teach that other sequencing methods which rely on electrophoresis are not well suited for large-scale genome projects or clinical sequencing where high throughput is needed (Column 1, lines 15-30). However the pyrosequencing method of Nyren opens up the possibility for an automated approach for large scale, non-electrophoretic sequencing procedures which allow for continuous

measurement of the progress of the polymerization reaction with time. The method of Nyren also has the advantage that multiple samples may be handled in parallel (Column 9, lines 4-6). Thus motivation is present and has been provided by Nyren. The fact that these reasons may be of secondary importance is only an opinion of the Appellants.

Appellants also note that an obviousness analysis starts with an analysis of the prior art, not from the claimed invention. The question is, whether it would have been obvious, at the time the invention was made, to combine and/or modify the prior art to arrive at the claimed invention. The Appellants point to a recent discussion of non-obviousness in Ortho-McNeil Pharmaceutical v. Mylan Labs, 2008-1223, Fed Cir. March 31, 2008. They note that in Ortho-McNeil the court specifically stated that the TSM test, flexibly applied merely assures that the obviousness test proceeds on the basis of evidence-teachings, suggestions, or motivations that arise before the time of invention as the statute requires. Appellants respectfully submit that the appropriate showing was not provided (pages 18-19 of the brief).

In response to Appellants argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In the instant case all of the claimed elements were known in the prior art.

Uhlmann and Nyren both teach methods for sequencing nucleic acids, an artisan of ordinary skill would have found it obvious to combine these teachings since they both pertain to sequencing. One of skill in the art would have been motivated to do so in order to achieve the benefits of real time sequencing which are discussed by Nyren.

B. Rejection of claims 12-16, 18 and 38 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al (Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568 Issued 2001) and in further view of Herman (U.S. Patent 5786146 Issued 1998).

C. Rejection of claim 17 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) and Herman (U.S. Patent 5786146 Issued 1998) as applied to claims 12 and 38 above, and in further view of Feinberg (Pub No. US 2003/0232351).

In the brief, regarding the rejections of B and C the Appellants state that each of the rejected claims depend from claim 12. The Appellants argue that claim 12 states "detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample to diagnose said pathological condition or the predisposition for said pathological condition". The Appellants argue that this language is presented in the body of the claim and not in the preamble. They note that this language does not constitute optional language. The also refer to the argument presented with respect to the independent claims (pages 19-20 in the brief).

These arguments have been considered but are not persuasive. It is noted for the record that claim 12 has been rejected under 35 USC 103(a) as being unpatentable over Uhlmann in view of Nyren AND as being unpatentable over Uhlmann in view of Nyren and in further view of Herman.

In the first rejection (over Uhlmann and Nyren) the phrase “to diagnose said pathological condition or the predisposition for said pathological condition” was interpreted as an intended use since the claim does not actually have an active process step of "diagnosing". The active process step of the method requires “determining whether said nucleotide is methylated or not methylated at said predetermined position”. Since the combined references teach this limitation the method of the combined references could be used to diagnose said pathological condition or the predisposition for said pathological condition particularly since Uhlmann teaches an association between hypomethylation and pilocytic astrocytomas and Nyren teaches pyrosequencing can be used to detect disease. The modification Uhlmann in view of Nyren does not change the other teachings of Uhlmann which state methylated DNA is associated with disease. The combination of Uhlmann and Nyren only changes how the sequence of a nucleic acid is determined.

In the second rejection (over Uhlmann, Nyren, and Herman) the phrase “to diagnose said pathological condition or the predisposition for said pathological condition” was given more patentable weight. The rejection states that neither Uhlmann nor Nyren teach that the methylation status is used to diagnose a pathological condition such as cancer, a neurodegenerative disease, or another neurological disorder. The

combined references do not teach that the primary tumor is a glioma selected from the group comprising: astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, and a pilocytic astrocytoma. The combined references also do not teach that the neurological disorder is selected from the group comprising: Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome. However, Herman teaches that the detection of methylated CpG containing nucleic acid is indicative of several disorders. Such disorders include but are not limited to low grade astrocytoma, anaplastic astrocytoma, glioblastoma, medulloblastoma, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, endometrial cancer and neuroblastoma. Identification of methylated CpG status is also useful for detection and diagnosis of genomic imprinting, fragile X syndrome and X-chromosome inactivation (Column 10, lines 49-58). So even if weight is given to the phrase "to diagnose said pathological condition or the predisposition for said pathological condition" in claim 12, Herman clearly teaches this.

The Appellants arguments regarding the independent claims (claims 1, 12, and 32) have been fully addressed above. The response to the Appellants arguments, as set forth above, applies equally to the present ground of rejection.

D. Rejection of claims 10, 25, 34, and 39 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claims 1 and 12 above, and in further view of Sylvan (US Patent 7078168 Filed 2/2002).

In the brief, regarding D the Appellants argue that Sylvan makes no reference to the methylation status of his population of nucleic acid molecules and certainly not "calculating a frequency of methylated nucleotides" as required by claims 10 and 25 Regarding the recitation of the phrase "can be" in claim 34 the Appellants argue that the phrase limits the claim. They state that the phrase "can be" in the context provided clearly states an ability that is either present or not. That is the method either can detect the allele frequency or not. This ability constitutes a limitation of the claim. The language is in the context provided, not conditional. Regarding claim 39 the Appellants state that the Office appears to concede that Sylvan does not exemplify a method wherein an allele frequency of 5% was detected with a standard deviation of not more than 1%. They state that the Office appears to believe that from Sylvans teachings there is an expectation that an allele frequency of 5% with a standard deviation of not more than 1% could be detected, and suggests that even if the expected results do not end up being equivalent to the actual results it would have been obvious to modify the method in order to determine the recited allele frequency. The Appellants argue that the examiner has not provided any reasoning why the person skilled in the art apart from the advantages of Sylvans method would make the combination to arrive at the claimed invention, namely to calculate the frequency of methylated nucleotides in particular with the accuracy set forth in the claims (see pages 20-22 of the brief).

These arguments have been fully considered but are not persuasive. Sylvan teaches a method of determining the frequency of an allele in a population of nucleic acid molecules (abstract). Specifically Figs 4a-c and Fig 6-7 depicts graphically relative

peak heights from a pyrosequencing reaction plotted against allele frequency. As you can see an allele frequency was detected. The wherein clause in claim 34 is conditional in view of the "can be" language. Therefore the claims actually only require detecting an allele frequency. Regarding claim 39, Sylvan does not exemplify a method wherein an allele frequency of 5% was actually detected with a standard deviation of not more than 1%, however Sylvan teaches that for SNPs SNPE1.5, SNPE7.5 and SNPE4.5 (See Figs 4a-c) they expected that an allele frequencies of 5% with a standard deviation of not more than 1% would be detected. Therefore even though the expected results were not equivalent to the obtained results, it would be obvious to modify the method in order to determine an allele frequency of 5% with a standard deviation of not more than 1%. It is noted that the pyrosequencing method of Nyren and Sylvan comprises all of the same pyrosequencing steps that are taught in the instant specification. Since the steps are all identical it is expected that any pyrosequencing method could achieve an allele frequency of 5% with a standard deviation of not more than 1%. Further as discussed in MPEP 2144.05(b), "(w)here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation. As such it would be obvious to optimize pyrosequencing and one of skill in the art would have a reasonable expectation of success since there are no differences between the pyrosequencing method of instant invention and the pyrosequencing method disclosed in the prior art.

E. Rejection of claim 35 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claim 1 above, and in further view of Laird (US 2002/0086324 Filed 10/2001).

F. Rejection of claim 37 under 35 U.S.C. 103(a) as being unpatentable over Uhlmann et al ((Electrophoresis 1999) in view of Nyren et al (U.S. Patent 6258568) as applied to claims 1 and 8 above, and in further view of Hyman (US 5602000 1997).

With regard to E and F the Appellants refer to the argument presented with respect to claim 1 since all of the rejected claims depend from claim 1.

The Appellants arguments regarding claim 1 have been fully addressed above. The response to Appellants arguments, as set forth above, applies equally to the present ground of rejection.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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